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Sequence-specific double-strand cleavage of DNA by Fe-bleomycin. 2. Mechanism and dynamics.

Absalon MJ, Wu W, Kozarich JW, Stubbe J.

Department of Chemistry, Massachusetts Institute of Technology, Cambridge 02139.

The mechanism of iron-bleomycin-mediated ds-cleavage of DNA has been investigated at specific sites within specific sequences using hairpin oligonucleotides (Absalon et al., 1995) and our recently developed technique for determining sequence-specific isotope effects upon oxidative degradation of DNA (Kozarich et al., 1989; Worth et al., 1993). Isotope effects upon ds-cleavage have been observed when the C-4' hydrogen of either nucleotide involved in the ds-break was substituted with deuterium. The values of the isotope effects determined for ss and ds events occurring at the same site were indistinguishable at four sites examined in detail. The results are consistent with a mechanism of ds-cleavage in which the pathways leading to ss- and ds-cleavage partition from a common intermediate subsequent to abstraction of the C-4' hydrogen from the first nucleotide involved in the cleavage. Deuterium substitution at the primary cleavage site of a ds-break failed to result in an equivalent effect on the amount of cleavage at the secondary cleavage site, suggesting that ds-cleavage may be initiated from either of the nucleotides involved in the ds-cleavage event. A kinetic preference for cleavage initiated at the 1 degree site, however, is probable. The requirement in the ds-cleavage process for O2, in addition to that needed to form "activated BLM", has been clearly demonstrated by the absence of ds-cleavage products in reactions performed under anaerobic conditions in which ss-cleavage still occurs. These results support, in part, the basic model for ds-cleavage proposed by Steighner and Povirk [(1990) Proc. Natl. Acad. Sci. U.S.A. 87, 8350-8354], in which a single molecule of BLM effects ds-cleavage and requires reactivation to effect cleavage at the second strand. The essential factor establishing the ratio of ss- to ds-cleavage at a specific site may be related to the efficiency by which Fe-BLM can be reactivated and/or repositioned at a second site for cleavage.

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Bioorganic & Medicinal Chemistry
Volume 3, Issue 6 , June 1995, Pages 839-849

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Enediyne-mediated cleavage of RNA

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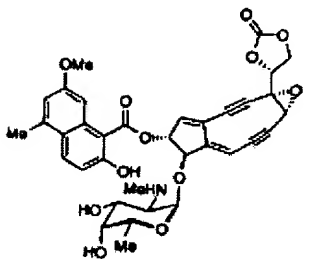
Received 3 January 1995; accepted 21 February 1995. ; Available online 14 February 2000.

Abstract

RNA cleavage by enediyne anticancer antibiotics was shown to occur with no apparent sequence selectivity, but RNA structure appears to be important in those substrates where cleavage was observed. Neocarzinostatin (NCS) cleaved a wider variety of RNA substrates than either esperamicin (ESP) or calicheamicin (CAL), and dynemicin (DYN) has yet to cleave any RNA substrate tried. NCS, ESP, and CAL were all observed to cleave RNA substrates near the 5'-end, and all three compounds exhibited cleavage in single-stranded loop regions of the RNA substrates. NCS required no thiol for activation and subsequent cleavage, but ESP and CAL required addition of thiol, as expected, for cleavage to occur. An RNA hairpin substrate containing a UCCU sequence, equivalent to the TCCT sequence preferred by CAL in double-stranded DNA substrates, was cleaved by CAL, but no retention of selectivity for the UCCU site was retained by CAL in this RNA substrate. This study confirms an earlier observation that RNA is a substrate for enediyne cleavage, and indicates that nucleic acid cleaving compounds such as the enediynes could be useful probes of RNA three-dimensional structure.

Graphical Abstract

Four enediynes were investigated for cleavage of structurally disctinct RNA molecules. Of the four enediynes tested, NCS cleaved a tRNA transcript, two hairpin RNAs, and a proposed pseudoknot RNA; CA1 and ESP cleaved two hairpin RNAs only; and DYN did not cleave any RNA molecule investigated.



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L15: Entry 2 of 2 File: USPT May 6, 2003

DOCUMENT-IDENTIFIER: US 6558509 B2
TITLE: Dual wafer load lock

Other Reference Publication (1):
Biggins, et al. "A continuous assay for DNA cleavage: The application of "break lights" to enediynes, iron-dependent agents, and nucleases"; PNAS. vol. 97, No. 25 13527-13542 (Dec. 2000).

Other Reference Publication (3):
Tyagi, et al. "Molecular Beacons: Probes that Fluoresce upon Hybridization"; Nature Biotechnology vol. 14, p. 303-308 (Mar. 1996).

L12: Entry 2 of 10 File: USPT Nov 13, 2001

DOCUMENT-IDENTIFIER: US 6316194 B1
TITLE: Methods and kits for discovery of RNA-binding antimicrobials

Brief Summary Text (7):
There are many different types of assays that measure the binding of ligands to nucleic acids and utilize fluorescence resonance energy transfer (FRET) to generate a signal. FRET is caused by a change in the distance separating a fluorescent donor group from an interacting resonance energy acceptor, either another fluorophore, a chromophore, or a quencher. Combinations of donor and acceptor moieties are known as "FRET pairs". Efficient FRET interactions require that the absorption and emission spectra of the dye pairs have a high degree of overlap. FRET is also a distance-dependent interaction which is dependent on the inverse sixth power of the intermolecular separation, making it a sensitive measurement of molecular distances (Stryer, 1978 and Selvin, 1995).

Brief Summary Text (34):
In certain preferred embodiments of the invention, only quenching of the donor due to the proximity of the acceptor in the antimicrobial/RNA complex is measured. In certain embodiments of the invention, the target RNA carries a chromophore or fluorophore that quenches the fluorescence of the fluorescent group on the antimicrobial after binding of the two molecules. In other embodiments of the invention, the antimicrobial carries a chromophore or fluorophore that quenches the fluorescence of the fluorescent group on the target RNA after binding of the two molecules.

Brief Summary Text (41):
As used herein, the term "donor" refers to a fluorophore which absorbs at a first wavelength and emits at a second, longer wavelength. The term "acceptor" refers to a fluorophore, chromophore or quencher with an absorption spectrum which overlaps the donor's emission spectrum and is able to absorb some or most of the emitted energy from the donor when it is near the donor group (typically between 1-100 nm). If the acceptor is a fluorophore capable of exhibiting FRET, it then re-emits at a third, still longer wavelength; if it is a chromophore or quencher, then it releases the energy absorbed from the donor without emitting a photon. Although the acceptor's absorption spectrum overlaps the donor's emission spectrum when the two groups are in proximity, this need not be the case for the spectra of the molecules when free in solution. Acceptors thus include fluorophores, chromophores or quenchers that, following attachment to either the RNA target molecule or to the antimicrobial, show alterations in absorption spectrum which permit the group to exhibit either FRET or quenching when placed in proximity to the donor through the binding interactions of two molecules.

Brief Summary Text (42):
As used herein, references to "fluorescence" or "fluorescent groups" or "fluorophores" include luminescence and luminescent groups, respectively.

Detailed Description Text (15):
Since oligoribonucleotides are sensitive to cleavage by cellular ribonucleases, as well as to alkaline or acid conditions, it may be preferable to use as the RNA target molecule a chemically modified molecule that mimics the action of the RNA binding sequence but is more stable. Other modifications may also be desirable to provide groups for immobilizing the RNA target oligonucleotide on solid supports by covalent or non-covalent attachments. The RNA target oligonucleotide may be a naturally

occurring oligonucleotide, or may be a structurally related variant of such an oligonucleotide having modified bases and/or sugars and/or linkages. The terms "RNA target" or "RNA target oligonucleotides" or "RNA oligonucleotides" as used herein are intended to cover all such variations.

Detailed Description Text (44):
The target RNA and the antimicrobial may be fluorescently labeled for use according to the invention by any suitable method, preferably by covalent attachment of a fluorescent group. The labels may be any fluorescent label or fluorophore that does not interfere with the ability of the antimicrobial to interact with the target RNA and is able to show quenching and/or fluorescence resonance energy transfer with the corresponding label on the target RNA.

Detailed Description Text (55):
Dehydrobutyrene and dehydroalanine moieties have characteristic reactions that can be utilized to introduce fluorophores, as illustrated but not limited to the following, where the fluorescent dye is conjugated to the indicated reactive group:

Detailed Description Text (57):
Useful fluorophores (in addition to those listed in Tables 5 and 6) include, but are not limited to: Texas Red.TM. (TR), Lissamine.TM. rhodamine B, Oregon Green.TM. 488 (2',7'-difluorofluorescein), carboxyrhodol and carboxyrhodamine, Oregon Green.TM. 500, 6-JOE (6-carboxy-4',5'-dichloro-2',7'-dimethyoxyfluorescein, eosin F3S (6-carobxymethylthio-2',4',5',7'-tetrabromo-trifluorofluorescein), cascade blue.TM. (CB), aminomethylcoumarin (AMC), pyrenes, dansyl chloride (5-dimethylaminonaphthalene-1-sulfonyl chloride) and other napththalenes, PyMPO, ITC (1-(3-isothiocyanatophenyl) -4-(5-(4-methoxyphenyl)oxazol-2-yl)pyridinium bromide).

Detailed Description Text (64):
Reference herein to "fluorescence"or "fluorescent groups" or "fluorophores" include luminescence, luminescent groups and suitable chromophores, respectively. In the present invention, the target RNA and antimicrobial may be labelled with luminescent labels and luminescence resonance energy transfer is indicative of complex formation. Suitable luminescent probes include, but are not limited to, the luminescent ions of europium and terbium introduced as lanthium chelates (Heyduk & Heyduk, 1997). The lanthanide ions are also good donors for energy transfer to fluorescent groups (Selvin, 1995). Luminescent groups containing lanthanide ions can be incorporated into nucleic acids utilizing an `open cage` chelator phosphoramidite. Table 5 gives some preferred luminescent groups.

Detailed Description Text (169):
Grasby, J. A., Butler, P. J. G. & Gait, M. J. (1993). The synthesis of oligoribonucleotides containing O6-methylguanosine: the role of conserved guanosine residues in hammerhead ribozyme cleavage. Nucl. Acids Res., 21, 4444-4450.

Detailed Description Text (173):
Heyduk, E. & Heyduk, T. (1997). Thiol-reactive, luminescent europium chelates: luminescence probes for resonance energy transfer distance measurements in biomolecules. Anal. Biochem., 248, 216-227.

Detailed Description Text (180):
Lamm, G. M., Blencowe, B. J., Sproat, B. S., Iribarren, A. M., Ryder, U. & Lamond, A. I. (1991). Antisense probes containing 2-aminoadenosine allow efficient depletion of U5 snRNP from HeLa splicing extracts. Nucl. Acids Res., 19, 3193-3198.

Detailed Description Text (204):
Rogers, J., Chang, A. H., von Ahsen, U., Schroeder, R. & Davies, J. (1996). Inhibition of the self-cleavage reaction of the human Hepatitis delta virus ribozyme by antibiotics. J. Mol. Biol., 259, 916-925.

Detailed Description Text (206):
Schmidt, S., Niemann, A., Krynetskaya, N. F., Oretskaya, T. S., Metelev, V. G., Suchomlinov, V. V., Shabarova, Z. A. & Cech, D. (1992). The use of oligonucleotide probes containing 2'-deoxy-2'-fluoronucleosides for regiospecific cleavage of RNA by RNase H from Escherichia coli. Biochim Biophys Acta, 1130, 41-46.

Detailed Description Text (213):
Sproat, B. S., Lamond, A. I., Beijer, B., Neuner, P. & Ryder, U. (1989). Highly efficient chemical synthesis of 2'-O-methyloligoribonucleotides and tetrabiotinylated derivatives; novel probes that are resistant to degradation by RNA or DNA specific nucleases. Nucl. Acids Res., 17, 3373-3386.

Detailed Description Text (216):
Sugiura, Y., Totsuka, R., Araki, M., Okuno, Y. (1997) Selective cleavages of tRNAPhe with secondary and tertiary structures by enediyne antitumor antibiotics. Bioorg. Med. Chem. 5, 1229-1234.

Detailed Description Text (217):
Sun, J. -S., Francois, J. -C., Lavery, R., Saison-Behmoaras, T., Montenay-Garestier, T., Thuong, N. T. & Helene, C. (1988). Sequence-targeted cleavage of nucleic acids by oligo-alpha-thymidylate-phenanthroline conjugates: parallel and antiparallel double helices are formed with DNA and RNA, respectively. Biochemistry, 27, 6039-6045.

Detailed Description Paragraph Table (9):
TABLE 5 Typical values of R.sub.o Donor Acceptor Ro (.ANG.)* Fluorescein Tetramethylrhodamine 55 IAEDANS Fluorescein 46 EDANS DABCYL 33 Fluorescein Fluorescein 44 BODIPY FL BODIPY FL 57 *R.sub.o is the distance at which 50% of excited donors are deactivated by FRET. Data from Haugland, RP. 1996. Handbook of Fluorescent Probes and Research Chemicals, 6th edition. Molecular Probes, Inc. Eugene OR, USA.

Detailed Description Paragraph Table (11):
TABLE 7 Antitumor antimicrobials acting on tRNA Antibiotic Target Reference Enediynes tRNA.sup.Phe Sugiura et al., 1997, Bioorg. Med. Chem. 5:1229 Colicin E5 (Nuclease) tRNA(Tyr, His, Asn, Asp), Ogawa et al., 1999, anticodon loop Science 283:2097

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L3	probe same (hairpin oligonucleotide or (stem loop near oligonucleotide))	22	L3
L4	l2 and fluorophore	225	L4
L5	l4 and quencher	112	L5
L6	L5 and enediyne	1	L6
L7	L5 and cleavage agent	10	L7
L8	L5 and bleomycin	0	L8
L9	enediyne	143	L9
L10	L9 and probe	49	L10
L11	L10 and fluorophore	10	L11
L12	L11 and cleav$	10	L12
L13	L7 and cleav$	10	L13
L14	molecular beacon probes	131	L14
L15	L14 and (bleomycin or enediyne)	2	L15
L16	L14 and l9	1	L16
L17	l14 and (fluorophore and quencher)	56	L17
L18	l17 and (hairpin oligonucleotide or stem loop oligonucleotide)	7	L18
L19	L18 and cleav$	4	L19

END OF SEARCH HISTORY